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ORIGINAL ARTICLE

Peptide Tyrosine Tyrosine 3-36 Reduces Meal Size and Activates the Enteric Neurons in Male Sprague–Dawley Rats

Kayla D. Newman[1] · Thaer R. Mhalhal[1,2] · Martha C. Washington[1] · John C. Heath[1] · Ayman I. Sayegh[1]

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Abstract
Background Peptide tyrosine tyrosine 3-36 (peptide YY 3-36 or PYY 3-36) reduces food intake by unknown site(s).
Aim To test the hypothesis that the gastrointestinal tract contains sites of action regulating meal size (MS) and intermeal interval (IMI) length by PYY 3-36.
Methods Peptide YY 3-36 (0, 1, 5, 10 and 20 nmol/kg) was injected in the aorta, the artery that supplies the gastrointestinal tract, prior to the onset of the dark cycle in free feeding male Sprague–Dawley rats and food intake was measured. Then, PYY 3-36 (25 nmol/kg) was injected intraperitoneally in these rats and Fos-like immunoreactivity (Fos-LI, a marker for neuronal activation) was quantified in the small intestinal enteric neurons, both myenteric and submucosal, and the dorsal vagal complex (DVC) of the hindbrain.
Results PYY 3-36 reduced first MS, decreased IMI length, shortened duration of first meal and increased Fos-LI in enteric and DVC neurons. However, PYY 3-36 failed to change the size of the second meal, satiety ratio, latency to first meal, number of meals and 24 h intake relative to saline control.
Conclusion The gastrointestinal tract may contain sites of action regulating MS reduction by PYY 3-36.

✉ Ayman I. Sayegh
sayeghai@mytu.tuskegee.edu

[1] Gastroenterology Laboratory, Department of Biomedical Sciences, College of Veterinary Medicine, Tuskegee University, Tuskegee, AL 36088, USA

[2] Department of Anatomy and Histology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq

Keywords Peptide tyrosine tyrosine 3-36 (PYY 3-36) · Fos-like immunoreactivity · Enteric nervous system · Aorta

Introduction

Peptide tyrosine tyrosine (PYY) is a 36 amino acid peptide [1] secreted by the L cells of the gastrointestinal (GI) tract in two molecular forms, PYY 1-36 and 3-36 [2–4]. However, due to rapid degradation by the enzyme dipeptidyl peptidase IV (DPP IV) PYY 1-36 is converted to PYY 3-36—the predominant circulating form of PYY.

Peptide YY 3-36 binds the neuropeptide Y2 receptor to evoke a number of responses. For example, it reduces gastric emptying and slows intestinal transit time, i.e., ileal brake [5–7]. In addition, it inhibits gastric acid secretion [8] and pancreatic exocrine secretion [9].

In 2002, Batterham et al. reported that intraperitoneal PYY 3-36 reduces food intake and increases Fos-like immunoreactivity (Fos-LI, a marker for neuronal activation) in the arcuate nucleus of the hypothalamus [10]. However, despite these results, the site of action for reduction in food intake by PYY 3-36 remained undetermined.

The current work tested the hypothesis that PYY 3-36 may reduce food intake through GI sites. This hypothesis stems from two findings. First, the major peripheral source of PYY 3-36 is the gut [1, 3, 9]. Second, the neuropeptide Y2 receptors, which mediates reduction in food intake by PYY 3-36, are distributed in the gastrointestinal tract [11, 12], the enteric neurons, vagal afferents [13, 14] and the central feeding control areas such as the arcuate nucleus and the nucleus tractus solitaries (NTS) [15, 16], which also connects with the GI tract through the vagus nerve.

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To test this hypothesis, we performed two experiments. First, we measured food intake in free feeding adult male Sprague–Dawley rats in response to PYY 3-36 given in the aorta, the artery that supplies the gut. Second, we quantified small intestinal (duodenum, jejunum and ileum) and dorsal vagal complex (DVC) neurons that express Fos-LI in response to intraperitoneal PYY 3-36. In support of the previous hypothesis, the results of the current work have shown that PYY 3-36 reduces meal size (MS) and increases Fos-LI in the enteric neurons of the small intestine.

Materials and Methods

Food Intake Study

Animals

The Tuskegee University Institutional Animal Care and Use Committee approved the protocols for all the experiments. Male Sprague–Dawley rats ($n = 8$, 450–550 g, Envigo, IN) were individually housed in the BioDAQ E2 system (Research Diets, New Brunswick, NJ) in a controlled environment (12 h dark/12 h light cycle—lights off at 1700 h, 21.5° C) with water and pelleted rodent chow (Teklad, Madison, WI) available ad libitum. To adapt the animals to the experimental protocol, each rat was handled daily for at least 10 min.

Vascular Catheterization

Each animal had one catheter implanted in the aorta as described previously [17–20]. Catheters were inserted in the aorta caudally to cranially (anally to orally), and the tip of each catheter was fixed before the origin of the cranial mesenteric artery (CMA).

Catheters (Micro-Renathane R-ITC-SP 9.5, Braintree Scientific, Braintree MA) were 24 cm long, the intravascular portion was 0.25 mm OD × 0.12 mm ID and the size of the remaining part was 0.84 mm OD × 0.36 mm ID. Catheterizations were performed using a surgical microscope (Carl Zeiss Opmi 160 12.5 ×/18B, 1 × 250, Monument, CO). General anesthesia, indicated by the absence of a pedal withdrawal reflex, was achieved with intramuscular injection of 1 ml/kg body weight of a mixture of 5.0 ml of Ketaset (100 mg/kg), 2.5 ml of Rompun® (xylazine 20 mg/kg), Bayer, Shawnee Mission, KS, 1.0 ml of acepromazine maleate® (10 mg/kg), Bayer, Shawnee Mission, KS and 1.5 ml of saline. The abdominal wall was clipped and cleaned with three alternating betadine solution and alcohol swabs. A ventral midline celiotomy was performed.

The aorta was exposed and two temporary ligations, 1 cm a part, were placed passing the origin of the CMA in the aborad direction to prevent bleeding. The aorta was punctured with a sterile 30-gauge needle between the two ligatures, and the catheter was threaded into the artery without blocking the entrance of the CMA. The catheter was fixed in place using cyanoacrylate glue, the temporary ligations were removed, and the catheter was threaded out of the abdominal cavity subcutaneously, exteriorized between the scapulae and secured with sutures and cyanoacrylate glue.

The muscles of the abdominal wall were closed using a polydioxanone II (4-0) absorbable suture in a simple continuous pattern, and the skin was closed using surgical staples. Postoperative care included Metacam® (Meloxicam® [1.1 mg/kg] Boehringer Ingelheim, St. Joseph, MO) subcutaneously for pain control, and Baytril® (Enrofloxacin® [0.05 ml], Bayer, Shawnee Mission, KS) intramuscularly as an anti-bacterial medication, each given daily for 5 d. Rats were allowed 2 weeks of recovery time. The criteria for complete recovery following surgery included the absence of clinical signs (e.g., signs of pain, porphyria secretion, cold extremities and lethargy) and the return of food intake to pre-operative levels. Catheters were flushed daily (1645 h) with 0.3 ml heparinized saline.

Meal Patterns

The BioDAQ E2 Food and Water Intake system detects brief episodes of food intake while minimizing food spillage and hoarding and generates a computerized data stream including times of the initiation of intake activity, the period of the activity, and the weight consumed. The criterion for a meal was consumption of $\geq$ 0.2 g, and the criterion for IMI was no feeding activity for $\geq$ 15 min [19–24].

Establishing Baseline Food Intake

Following recovery, rats were habituated to the laboratory environment and experimental design daily for 2 weeks. At 0900 h, the rats were weighed and at 1645 h they received a 0.3 ml infusion of heparinized saline in their catheters. At 1700 h, lights were off and meal size (MS), intermeal interval (IMI), satiety ratio (SR), latency to the first meal, duration of first meal, number of meals and 24-h total intake were determined and formed individual baselines for each of the rats. These were compared later with the experimental data (saline treatments). If they did not match within two standard deviations difference, they were not included in the statistical analysis. All rats were included in the analysis.

Food Intake Experiment

After we established the baseline intake for all animals, infusion of the peptides or saline vehicle started. Rats were weighed at 0900 h every day. At 1645 h each rat received an infusion of PYY 3-36 (1, 5, 10 and 20 nmol/kg) (Bachem, CA) or vehicle (saline) every other day for 9 days. Immediately following the infusion(s), at 1700 h, normal rat chow and water were available and remained in the cages at all times.

Statistical Analysis

The data for each response, e.g., MS, IMI were analyzed using repeated measures design and a general linear model post hoc test for pairwise comparisons. A *t*-test for pairwise comparisons to compare the response between the dark and the light cycles was also used. Results were considered significant if $p < 0.05$. Data are displayed as mean ± standard error of the mean (SEM).

Immunohistochemistry

Animals

The Tuskegee University Animal Care and Use Committee approved all of the animal protocols for this study. Adult, male Sprague–Dawley rats ($n = 14$ total; 8 injected with PYY 3-36, 2 injected with CCK-8 and 4 injected with saline) weighing between 460 and 520 g and housed individually in clear, plastic cages were used in this study. The rats lived in a controlled environment (12 h dark/12 h light cycle, with lights off at 1700 h, temperature at 21.5 °C and ad libitum water and pelleted rodent chow, Teklad, WI).

To habituate the rats to the laboratory environment and experimental design, each rat was weighed, handled for 10 min and received an intraperitoneal (i.p.) injection of saline at the same time every day. All injections were delivered in a volume of 0.5 ml and were given at 0700 h, which was 2 h into the beginning of the light cycle to allow time for the preparation of the injections and materials.

Experimental Procedure

At the time of the experiment, rats were fasted from food, but not water, beginning at 1700 h on the day prior to all experiments. The following morning, the rats received CCK-8 (100 nmol/kg), PYY 3-36 (25 nmol/kg) (Bachem, CA) or saline vehicle i.p. The choice of this dose was based on our previous work, which demonstrated a significant reduction in food intake with PYY 3-36.

Ninety minutes after the injections, the optimum time for Fos expression was determined in our previous studies [25–27]. All rats were killed with an overdose of sodium pentobarbital (10 mg/kg, i.p.) and perfused transcardially in two stages: first, with 500 ml of Krebs solution to collect the intestine, and second, with 500 ml of 4% formaldehyde solution dissolved in 0.1 M phosphate-buffered saline (PBS) to collect and fix the brains in situ.

Five-centimeter segments of small intestine were collected between 5 and 10 cm aborad from the pylorus (duodenum), between 20 and 25 cm aborad from the pylorus (jejunum) and between 5 and 10 cm orad from the cecum (ileum). The intestinal whole mounts (duodenum, jejunum, and ileum) were collected through a ventral midline celiotomy incision and prepared for detection of Fos-LI in the myenteric and submucosal plexuses, as described previously [25–27].

The brainstems were also collected and postfixed with 4% formaldehyde for 2 h at 4 °C and then placed in a 25% sucrose solution overnight at room temperature. On the following day, the brainstems were sectioned in 40 Âμm sections on a cryostat at − 20 °C, blocked with 50% ethyl alcohol for 30 min to reduce the production of free radicals and improve background staining, and processed to detect the Fos-LI as described elsewhere [25–27]. Consistent with those and other studies, the areas of the DVC included the AP (− 4.5 mm caudal to interaural plane), NTS (− 4.5 and − 4.8 mm caudal to interaural plane) and DMV (− 4.5 and − 4.8 mm caudal to interaural plane) according to the Rat Brain Atlas (Paxinos G., 1997). The sections were taken from multiple levels of the NTS to insure sampling from sites receiving both gastric and intestinal vagal afferent innervation.

The intestinal whole mounts and the brain sections were incubated for 24 h at room temperature in a primary antiserum raised in rabbit against a peptide consisting of amino acids 4–17 of human Fos (Oncogene, Ab-5, San Diego, CA [1:12,000 dilution]). On the next day, the tissues were rinsed with 0.01 M tris phosphate-buffered solution (TPBS) and incubated overnight in biotinylated donkey anti-rabbit serum (Jackson ImmunoResearch Laboratories, PA [1:500 dilution]). The tissues were incubated for 3 h in avidin conjugated to horseradish peroxidase, washed with 0.01 M TPBS, and reacted with glucose oxidase and diaminobenzidine (DAB, Sigma, MO) intensified with nickel.

Counting Procedure

With the slide labels concealed, two examiners who were blinded to the treatments of the experiment counted and averaged the counts of Fos-positive neurons in the enteric neurons in 20 non-overlapping, microscopic fields using

Q-Imaging software and a 100-magnification lens. The criteria included only black, rounded, regular nuclei. All large, irregular, faintly stained shapes were considered cell bodies and were not counted. The agreement rate between the two examiners exceeded 98%. In the DVC, the Fos-positive neurons were counted by an automated computer software (ImagePro Plus, Media Cybernetics) in the AP, NTS, and DMV.

Statistical Analysis

The counts of the Fos-positive neurons in the DVC were compared using a two-way analysis of variance (ANOVA) (treatment and level were the two independent variables) followed by the Tukey's test for multiple comparisons. The counts of the Fos-positive neurons in the intestines were compared using a three-way analysis of variance (ANOVA) (treatment, section and layer were the independent variables) followed by the Tukey's test for multiple comparisons. The results are displayed as mean ± SEM, and counts were significant if $p < 0.05$.

Results

First Meal Size

PYY (3-36) (5, 10 and 20 nmol/kg) given in the aorta reduced the first nocturnal meal size relative to saline ($F(4, 24) = 9.948$, $p = 0.021$, 0.006, 0.001, respectively, $\eta^2 = 0.624$). PYY (3-36) 10 nmol/kg reduced first meal more than 5 nmol/kg ($p = 0.013$) (Fig. 1).

Second Meal Size

PYY (3-36) (0, 1, 5, 10 and 20 nmol/kg) given in the aorta had no effect on the second nocturnal meal size relative to saline ($p = 0.373$, 0.251, 0.076, and 0.470, respectively).

First Intermeal Interval

PYY (3-36) (10 nmol/kg) given in the aorta decreased the first IMI relative to saline vehicle ($F(4, 24) = 3.553$, $p = 0.016$, $\eta^2 = 0.372$) (Fig. 2).

First Satiety Ratio

PYY (3-36) (1, 5, 10 and 20 nmol/kg each) given in the aorta had no effect on the first SR relative to saline vehicle ($p = 0.801$, 0.159, 0.893, 0.181, respectively).

Latency to the First Meal

PYY (3-36) (1, 5, 10 and 20 nmol/kg) given in the aorta failed to significantly increase the latency to the first meal relative to saline vehicle ($p = 0.224$, 0.368, 0.384, 0.115, respectively).

Duration of the First Meal

PYY (3-36) (10 nmol/kg) given in the aorta significantly decreased the duration of the first meal relative to saline vehicle ($F(4, 16) = 2.273$, $p = 0.05$, $\eta^2 = 0.362$) (Fig. 3).

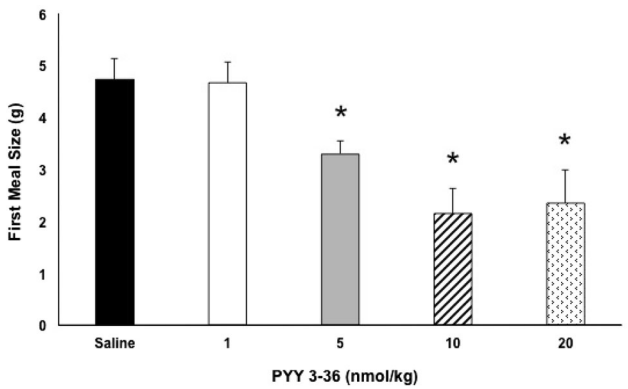

Fig. 1 Effect of peptide tyrosine tyrosine 3-36 on the size of the first meal. Peptide YY 3-36 (0, 1, 5, 10 and 20 nmol/kg) was infused in the aorta prior the onset of the dark cycle and the first meal size (MS, normal rat chow) was determined. Peptide YY 3-36 (5, 10 and 20 nmol/kg) reduced MS relative to saline vehicle (*, $p < 0.05$)

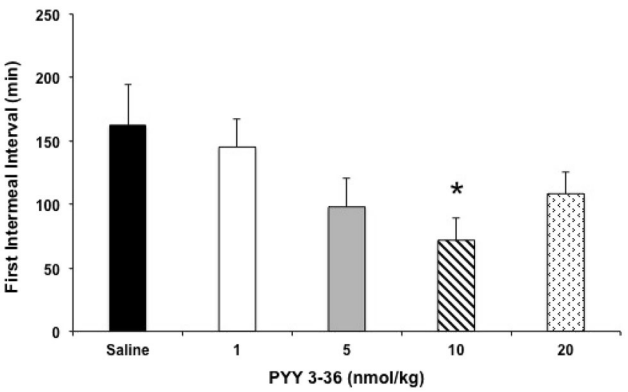

Fig. 2 Effect of peptide tyrosine tyrosine 3-36 on the first intermeal interval. Peptide YY 3-36 (0, 1, 5, 10 and 20 nmol/kg) was infused in the aorta prior the onset of the dark cycle and the first intermeal interval (IMI, time between first and second meal) was determined. Peptide YY 3-36 10 nmol/kg reduced the IMI relative to saline vehicle

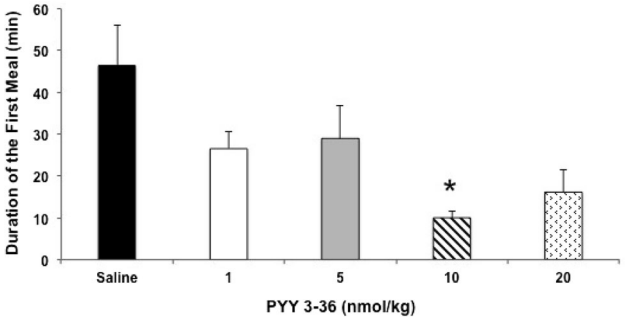

Fig. 3 Effect of peptide tyrosine tyrosine 3-36 on the duration of the first meal. Peptide YY 3-36 (0, 1, 5, 10 and 20 nmol/kg) was infused in the aorta prior the onset of the dark cycle and the duration of the first meal was determined. Peptide YY 3-36 10 nmol/kg shortened this duration relative to saline vehicle

Number of Meals

PYY (3-36) (1, 5, 10 and 20 nmol/kg) given in the aorta had no effect on the number of meals relative to saline vehicle (p = 0.663, 0.584, 0.351, 0.351, respectively).

24 h Intake

PYY (3-36) (1, 5, 10 and 20 nmol/kg) given in the aorta had no effect on 24 h intake relative to saline vehicle (p = 0.662, 0.704, 0.840, 0.949, respectively).

First Meal Size During Light and Dark Cycles

The size of the first meal during the light and dark cycles was compared for both PYY 3-36 and saline vehicle control. The first meal size in the light cycle was significantly different when compared to the dark cycle in the saline vehicle control and the PYY 3-36 1 nmol/kg dose (p = 0.012, 0.001, respectively) (Fig. 4).

Intermeal Interval Length During Light and Dark Cycles

The first intermeal interval during the light and dark cycles was compared for both PYY 3-36 and saline vehicle control. There was no difference in the IMI length between the light and dark cycles (Fig. 5).

Effect of PYY 3-36 on Activation of the Dorsal Vagal Complex (DVC)

Figure 6 shows photomicrographs of the DVC at − 4.5 mm caudal to the interaural plane captured from a saline treated rat (Fig. 6a), PYY 3-36 (25 nmol/kg)-treated rat (Fig. 6b) and CCK-8 (100 μg/kg)-treated rat (Fig. 6c). PYY 3-36 and CCK-8 increased Fos-LI more than in saline

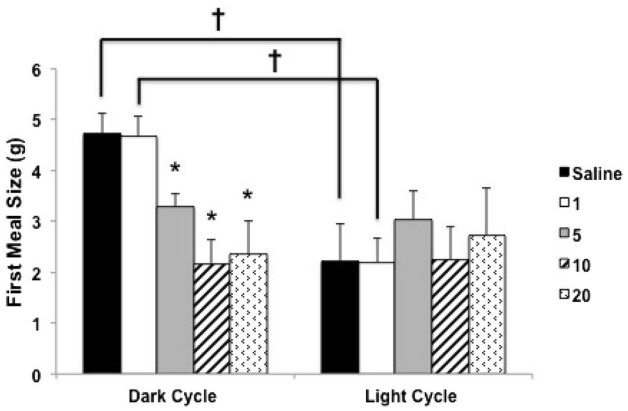

Fig. 4 Effect of peptide tyrosine tyrosine 3-36 on the average first meal size during the dark and light cycles. Peptide YY 3-36 (0, 1, 5, 10 and 20 nmol/kg) was infused in the aorta prior the onset of the dark cycle and first MS was measured. During the dark cycle, PYY 3-36 (5, 10, and 20 nmol/kg) reduced MS relative to saline vehicle (*, $p < 0.05$). During the light cycle, PYY 3-36 1 nmol/kg and saline vehicle was significantly less when compared to the dark cycle (†, $p < 0.05$)

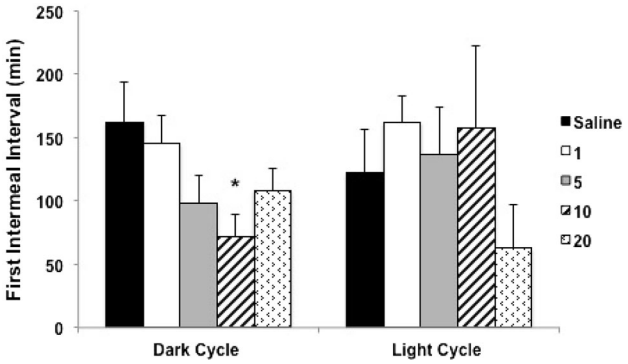

Fig. 5 Effect of peptide tyrosine tyrosine 3-36 on the average intermeal interval during the dark and light cycles. Peptide YY 3-36 (0, 1, 5, 10 and 20 nmol/kg) was infused in the aorta prior the onset of the dark cycle and the first intermeal interval (IMI, time between first and second meal) was determined. During the dark cycle, PYY 3-36 10 nmol/kg reduced the IMI relative to saline vehicle. During the light cycle, there was no difference in IMI lengths when compared to the dark cycle

in all areas of the DVC. In the AP, PYY 3-36 (25 nmol/kg) and CCK-8 (100 μg/kg) increased the expression of Fos-LI compared to saline ($p < 0.001$).In the NTS and DMV (− 4.5 mm and − 4.8 mm caudal to interaural plane), PYY 3-36 (25 nmol/kg) and CCK-8 (100 μg/kg) increased the expression of Fos-LI compared to saline ($p < 0.001$) (Fig. 7).

Effect of PYY 3-36 on Activation of the Enteric Neurons

Figure 8 is a photomicrograph of both the myenteric and submucosal plexus of the duodenum, captured from saline

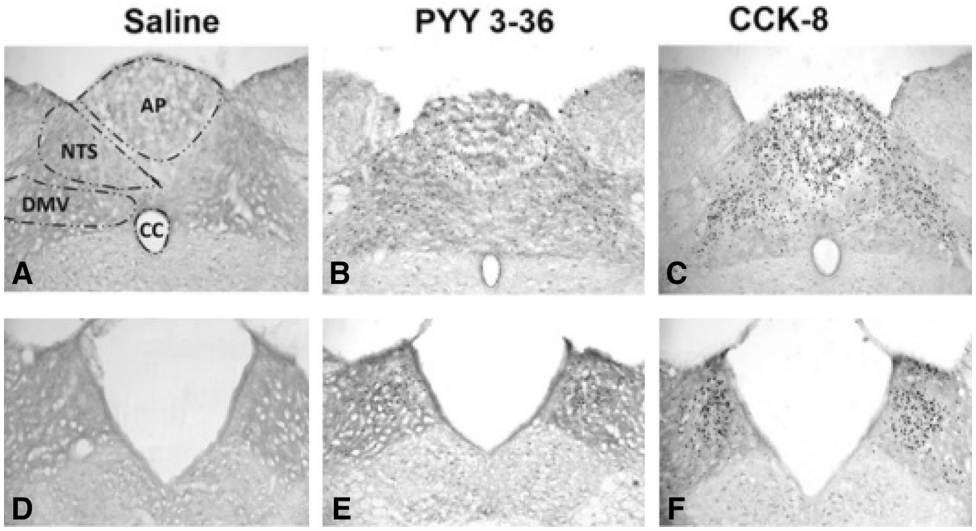

Fig. 6 Effect of peptide tyrosine tyrosine 3-36 on Fos-like immunoreactivity in the dorsal vagal complex. Photomicrographs of dorsal vagal complexes obtained from rats injected with saline (**a**, **d**), peptide YY 3-36 (PYY 3-36) (**b**, **e**), and cholecystokinin-8 (CCK-8) (**c**, **f**). PYY 3-36 and CCK-8 increased Fos-like immunoreactivity, seen as dark pin-point dots, relative to saline in the area postrema (AP), nucleus tractus solitaries (NTS), and dorsal motor nucleus of the vagus (DMV). Two levels were taken at − 4.5 mm caudal to interaural plane (**a**–**c**) and − 4.8 mm caudal to interaural plane (**d**–**f**)

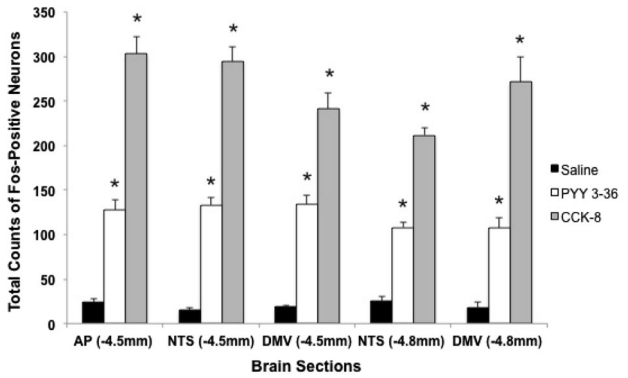

Fig. 7 Effect of peptide tyrosine tyrosine 3-36 on activation of the dorsal vagal complex. PYY 3-36 (25 nmol/kg, white bars) and CCK-8 (100 ug/kg, gray bars) or saline (black bars) were given i.p. to overnight food-deprived male Sprague–Dawley rats [($n = 14$) 8 PYY 3-36, 2 CCK-8, and 4 Saline]. Fos-like immunoreactivity (Fos-LI) was determined, and the activated neurons were quantified in five different areas of the dorsal vagal complex of the hindbrain: area postrema (AP), and nucleus tractus solitaries (NTS) and dorsal motor nucleus of the vagus (DMV) at two levels − 4.5 and − 4.8 mm (1 and 2, respectively) caudal to interaural plane. PYY 3-36 and CCK-8 activated the AP, NTS and DMV relative to saline (*, $p < 0.05$)

treated rat (Fig. 8a, b), PYY 3-36 (25 nmol/kg)-treated rat (Fig. 8g, h) and CCK-8 (100 ug/kg)-treated rat (Fig. 8m, n). PYY 3-36 and CCK-8 increased Fos-LI more than in saline in both plexuses. In the myenteric plexus of the jejunum, PYY 3-36 (Fig. 8i) and CCK-8 (Fig. 8o) increased Fos-LI expression compared to saline (Fig. 8c) ($p = 0.008$ and $p = 0.002$, respectively). PYY 3-36 and CCK-8 did not increase Fos-LI in the myenteric of the ileum or the submucosal of the jejunum and ileum (Fig. 9).

Discussion

The results of the current study support the hypothesis that the gastrointestinal tract may contain sites of action controlling MS reduction by PYY 3-36. Here, we found that intra-arterial injections of PYY 3-36 in the aorta, the artery that supplies the gut, reduce MS. In addition, we have also found that ip injections of the same peptide increase Fos-LI in the enteric neurons and in feeding control areas in the DVC.

Reduction in food intake by PYY 3-36 has been documented previously [10, 28–32]. However, the site of action for this reduction remains unknown. It has been proposed that following its release from the L cells of the gut PYY 3-36 reduces food intake by two methods. (A) PYY 3-36 can directly activate neuropeptide Y2 receptor in the central feeding areas of the forebrain, e.g., arcuate nucleus of the hypothalamus and/or the hindbrain, e.g., DVC, especially areas that lack the blood brain barrier, e.g., area postrema. (B) PYY 3-36 can indirectly activate the same receptors by means of stimulating gastrointestinal or hepatic vagal afferents [10, 15, 32–37], which synapse with the previous central feeding areas.

The results of the current study provide two lines of evidence that suggest, but does not confirm, a gastrointestinal site of action for reduction of food intake by PYY 3-36. First, PYY 3-36 reduced food intake following intra-aortic infusions. The aorta is the only artery that supplies the gut. Second, PYY 3-36 activated the neurons of the DVC as shown previously [15, 35, 38, 39] as well as the enteric, both myenteric and submucosal, neurons of the

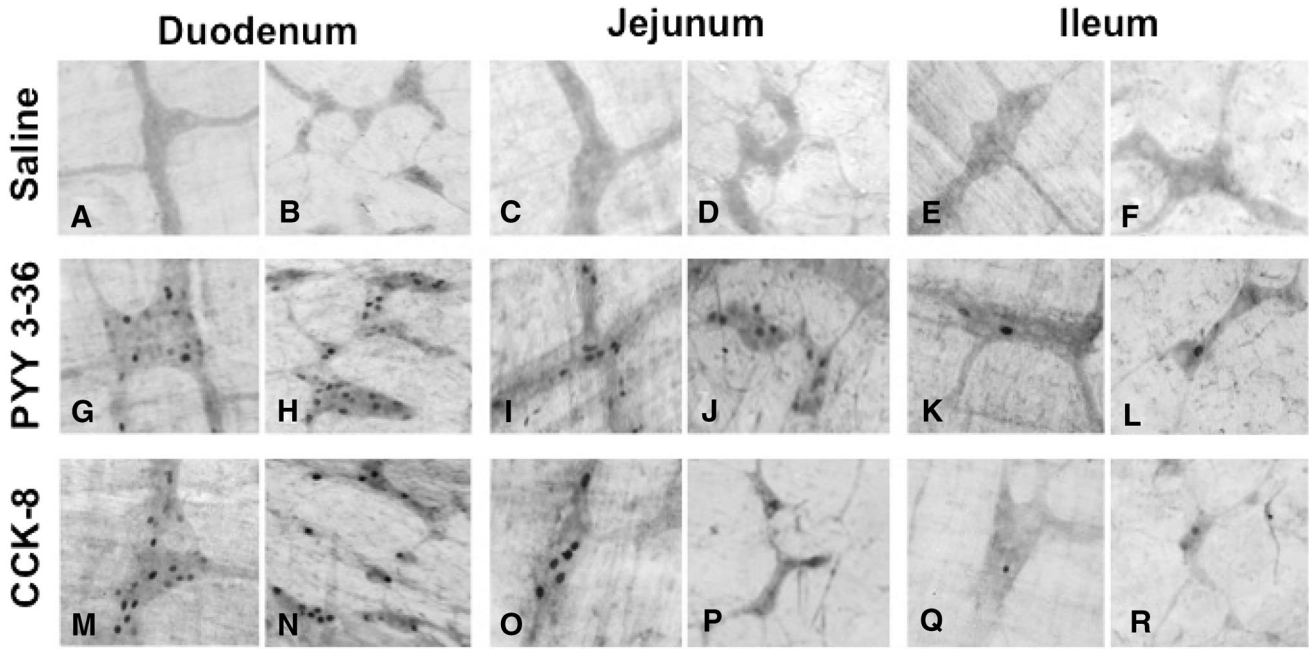

Fig. 8 Effect of peptide tyrosine tyrosine 3-36 on Fos-like immunoreactivity in the enteric nervous system of the small intestine. Photomicrographs of duodenum myenteric neurons (**a**, **g**, **m**) and duodenum submucosal neurons (**b**, **h**, **n**), jejunum myenteric neurons (**c**, **i**, **o**) and jejunum submucosal neurons (**d**, **j**, **p**), ileum myenteric neurons (**e**, **k**, **q**) and ileum submucosal neurons (**f**, **l**, **r**), captured from rats injected with saline (**a**–**f**), peptide YY 3-36 (PYY 3-36) (**g**–**l**), and cholecystokinin-8 (CCK-8) (**m**–**r**). PYY 3-36 and CCK-8 increased Fos-like immunoreactivity in the myenteric and submucosal neurons of the duodenum (**g**, **h**, **m**, **n**) and PYY 3-36 and CCK-8 increased it in the myenteric plexus of the jejunum (**i**, **o**)

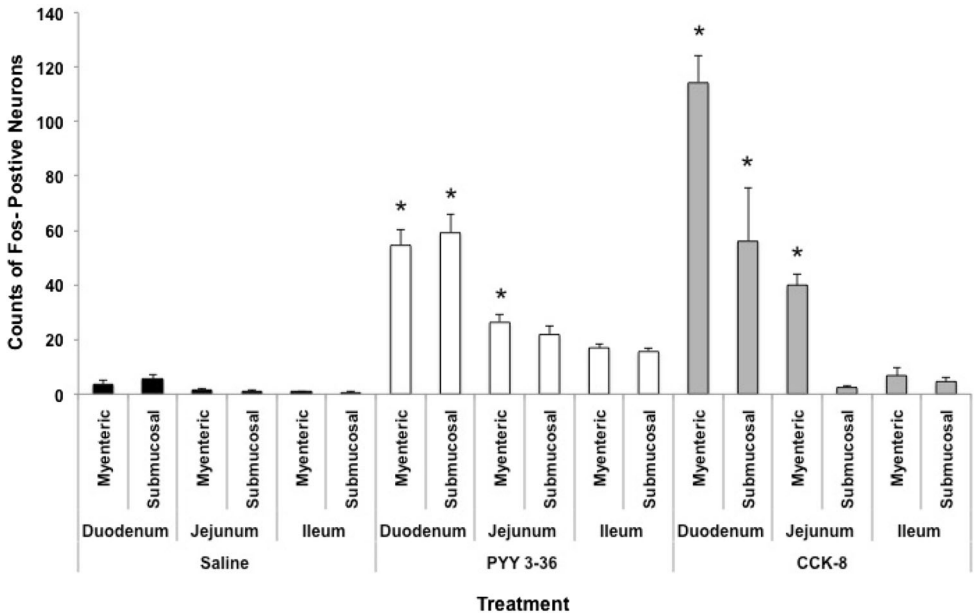

Fig. 9 Effect of peptide tyrosine tyrosine 3-36 on Fos-like immunoreactivity in the enteric nervous system of the small intestine. Peptide YY 3-36 (25 nmol/kg, white bars), cholecystokinin-8 (100 ug/kg, gray bars) or saline (black bars) were given i.p. to overnight food-deprived male Sprague–Dawley rats [($n = 14$) 8 PYY 3-36, 2 CCK-8, and 4 Saline]. Fos-like immunoreactivity (Fos-LI) was determined, and the activated neurons were quantified in the myenteric and submucosal neurons of the duodenum, jejunum and ileum. PYY 3-36 and CCK-8 activated the myenteric and submucosal neurons of the duodenum relative to saline (*), and PYY 3-36 and CCK-8 activated the myenteric neurons of the jejunum relative to saline (*, $p < 0.05$)

small intestine. Therefore, the gastrointestinal tract may contain sites of action controlling reduction of food intake by PYY 3-36. However, there are limitations in this study. For example, the routes of infusion in the two experiments were different. Future work must examine food intake and activation of enteric and central neurons by PYY 3-36 using only the intra-arterial infusion route to determine whether the gastrointestinal tract contains sites of action regulating MS and IMI length by PYY 3-36. Furthermore, mechanistic studies, e.g., antagonist against the Y2 receptor, truncal vagotomy, celiaco-mesenteric ganglionectomy, intestinal myotomy or combination of these methods are necessary to confirm a possible role for the gastrointestinal tract in containing sites of action controlling MS and IMI length by PYY 3-36.

We propose that reduction of food intake by PYY 3-36 may take place by the following pathway. Nutrients stimulate the release of PYY 3-36 from the endocrine L cells of the gut. This in turn activates local enteric neurons, both myenteric and submucosal, which synapse with vagal afferents that contain Y2 receptors and activate the central feeding control areas in the DVC to reduce food intake. However, as stated earlier (please see above), future studies are required to confirm not only the plausible role of the gut in having sites of action regulating MS and IMI length by PYY 3-36, but also for a possible role of the extrinsic innervation of the gut in carrying the satiety signal by this peptide centrally. This is especially important because the Y2 receptor has a wide range of tissue distribution both peripherally and centrally.

However, the gastrointestinal tract remains a potential site of action for reduction of food intake by PYY 3-36 for at least three main reasons. (A) The gut is the major peripheral source of PYY 3-36, which also contains Y2 receptors that are necessary for reduction of food intake by PYY 3-36 [11, 40–42]. (B) The enteric neurons are activated by PYY 3-36 (current study). (C) The vagal afferents, which synapse with the enteric neurons and activate the central feeding control areas in the forebrain and the hindbrain, also contain Y2 receptors [43–46]. Again, this plausibility requires further testing as indicated previously.

Using a similar experimental design as the one used in the first experiment of the current study, we have shown that the gastrointestinal tract may have sites of action regulating food intake reduction by other gut peptides. For example, intra-arterial infusions of cholecystokinin, gastrin releasing peptide and glucagon like peptide-1 reduced MS and prolonged IMI length [19–24], and the same peptides increased Fos-LI in the enteric neurons [25, 26, 47] when given intraperitoneally. Future studies are needed to quantify Fos-LI in the enteric and DVC neurons following intra-arterial injection of the peptides, including PYY 3-36. Furthermore, to identify the specific gastrointestinal site(s) of action that regulates reduction of MS by PYY 3-36, future studies must include delivering the peptide by the branches of the aorta that supply the different portions of the gut, i.e., celiac artery, supplying stomach and upper small intestine, and cranial mesenteric artery, supplying small and part of the large intestine.

In conclusion, the results of the current experiments suggest, but does not confirm, that the gastrointestinal tract may have a role in the reduction of food intake evoked by PYY 3-36. Further studies are required to confirm such conclusion.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

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